CANADA COMMUNICABLE DISEASE REPORT

CCDR

40 years! (1975-2015)

August 6, 2015 • VOLUME 41•8
ISSN 1481-8531

Inside this issue: Protein misfolding disorders

This issue explores a rapidly developing area of research: protein misfolding disorders. It was once thought that prions, which caused protein misfolding in "Mad Cow Disease" and other forms of Creutzfeldt-Jakob disease (CJD), represented a unique disease process. It is now becoming apparent that "prion-like" disorders may be linked to a number of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Read the first published surveillance report on CJD in Canada, and learn about advances in both diagnostics and future therapies.

Surveillance

Creutzfeldt-Jakob disease mortality in Canada, 1998–2013 182
Coulthart MB, Jansen GH, Connolly T, D'Amour R, Kruse J, Lynch J et al

Innovation

A new diagnostic test for Creutzfeldt Jakob disease (CJD): Real-Time Quaking Induced Conversion (RT-QuIC) 192
Godal D, Simon SL, Cheng K, Knox JD

Commentary

Protein misfolding: new opportunities for therapeutics, new public health risk 196
Cashman NR

ID News

Protein misfolding 200

Upcoming conferences/education

August 24-26, 2015: International Conference on Emerging Infectious Diseases. Atlanta, United States.
http://www.iceid.org/

September 17-21, 2015: Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC) San Diego, United States.
http://www.icaac.org/index.php/2015-01-26-21-25-32/meeting/icaac-icc-2015

November 11-13, 2015: European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE). Stockholm, Sweden.
http://ecdc.europa.eu/en/escaide/Pages/ESCAIDE.aspx

Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Canada Communicable Disease Report

The *Canada Communicable Disease Report* (CCDR) is a bilingual, peer-reviewed, open-access online scientific journal published by the Public Health Agency of Canada (PHAC). It provides timely, authoritative and practical information on infectious diseases to clinicians, public-health professionals, and policy-makers to inform policy, program development and practice.

Editorial Office

Patricia Huston, MD, MPH
Editor-in-Chief

Mylène Poulin, BSc, BA
A/Managing Editor

Jacqueline Smith
Production Editor
613-851-5138

Diane Staynor
Editorial Assistant
613-851-5033

Diane Finkle-Perazzo
Cathy Robinson
Copy Editors

CCDR Editorial Board

Michel Deilgat, CD, BA, MD, CCPE
Centre for Foodborne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada

Catherine Dickson, MDCM, MSc
Resident, Public Health and Preventive Medicine
University of Ottawa

Juliana Fracassi, RN, BScN
Infectious Diseases
Ottawa Public Health

Jennifer Geduld, MHSc
Centre for Foodborne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada

Judy Greig, RN, BSc, MSc
Laboratory for Foodborne Zoonoses
Public Health Agency of Canada

Judy Inglis, BSc, MLS
Office of the Chief Science Officer
Public Health Agency of Canada

Mohamed A. Karmali, MB ChB, FRCPC
Infectious Disease Prevention and Control Branch
Public Health Agency of Canada

Julie McGihon
Public Health Strategic Communications Directorate
Public Health Agency of Canada

Robert Pless, MD, MSc
Centre for Immunization and Respiratory Infectious Diseases, Public Health Agency of Canada

Hilary Robinson, MB ChB, MSc, FRCPC
Health Security Infrastructure Branch
Public Health Agency of Canada

Rob Stirling, MD, MSc, MHSc, FRCPC
Centre for Immunization and Respiratory Infectious Diseases, Public Health Agency of Canada

Jun Wu, PhD
Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada

Canada Communicable Disease Report
Public Health Agency of Canada
130 Colonnade Rd.
Address Locator 6503B
Ottawa, Ontario K1A 0K9
Email: CCDR-RMTC@phac-aspc.gc.ca

To promote and protect the health of Canadians through leadership, partnership, innovation and action in public health.
Public Health Agency of Canada

Published by authority of the Minister of Health.
© Her Majesty the Queen in Right of Canada, represented by the Minister of Health, 2015
ISSN 1481-8531
Pub.150005

This publication is also available online at http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/15vol41/index-eng.php
Également disponible en français sous le titre: *Relevé des maladies transmissibles au Canada*

Surveillance

Creutzfeldt-Jakob disease mortality in Canada, 1998 to 2013

Coulthart MB[1*], Jansen GH[1,2], Connolly T[1], D'Amour R[1], Kruse J[1], Lynch J[1], Sabourin S[1], Wang Z[1†], Giulivi A[2], Ricketts MN[3], Cashman NR[4]

[1]Canadian Creutzfeldt-Jakob Disease Surveillance System, Centre for Foodborne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada, Ottawa, ON
[2]The Ottawa Hospital, University of Ottawa and Eastern Ontario Regional Laboratory Association, Ottawa, ON
[3]University of Ottawa, Ottawa, ON
[4]Department of Medicine (Neurology), Brain Research Centre, University of British Columbia, Vancouver, BC
[†]Deceased November 2014

*Correspondence: michael.coulthart@phac-aspc.gc.ca

The authors dedicate this paper to the memory of their friend and colleague Zheng Wang (1962–2014).

Abstract

Background: Human prion diseases, known collectively as Creutzfeldt-Jakob disease (CJD), are fatal, infectious neurodegenerative disorders that occur in all human populations.

Objective: To summarize national surveillance data for CJD in Canada between January 1, 1998, and December 31, 2013.

Methods: Detailed investigations were conducted of individual suspected CJD cases, with collaboration between Canadian health professionals and investigators affiliated with a central CJD surveillance registry operated by the Public Health Agency of Canada. Data were collected on the clinical profile, family history, and results of paraclinical and laboratory investigations, including post-mortem neuropathological examination.

Results: A total of 662 deaths from definite and probable CJD were identified in Canadian residents during the study period, comprising 613 cases of sporadic CJD (92.6%), 43 cases of genetic prion disease (6.5%), 4 cases of iatrogenic CJD (0.6%), and 2 cases of variant CJD disease (0.3%). The overall crude mortality rate for sporadic CJD was 1.18 per million per year [95% confidence interval (CI): 1.08,1.27]. Age-specific rates ranged from 0.05 [95% CI: 0.03,0.08] in persons under 50 years of age to 7.11 [95% CI: 6.20,8.11] in those aged 70 to 79. A significant net upward trend in age-adjusted rates was observed over the study period. Standardized mortality ratios, calculated for 10 individual Canadian provinces with reference to national average mortality rates, did not differ significantly from 1.0.

Conclusion: Creutzfeldt-Jakob disease remains rare in Canada, although mortality rates vary by two orders of magnitude between older and younger age groups. The upward trend in age-standardized sporadic CJD mortality rate over the study period can be better accounted for by gradually improving case ascertainment than by a real increase in incidence.

Introduction

Prion diseases are rare, lethal, degenerative brain disorders that affect humans and several other mammals. Remarkably, these diseases are transmissible by an infection-like molecular process involving a pathologically altered, self-propagating form of a host glycoprotein, the prion protein (PrP). The infective agents of these diseases are therefore referred to as prions, or proteinaceous infectious particles (1). Despite recently recognized similarities between the molecular mechanisms of prion diseases linked with pathological behaviour

of PrP and other "prion-like" diseases involving misfolding of other proteins, to date there is no evidence as yet that prion-like diseases are transmissible between individuals under natural conditions (2,3). Thus, prion diseases pose a unique combination of challenges to public health, including long, silent incubation periods; lack of protective host immunity; resistance to decontamination; limited tools for diagnosis; and potential for both zoonotic and health care-based transmission (4).

Prion diseases of humans—for brevity, referred to collectively here as Creutzfeldt-Jakob disease (CJD)—exhibit various clinicopathological presentations that reflect distinct endogenous and exogenous etiologic origins. Among "classic" forms of CJD the most common subtype, sporadic CJD (sCJD), is defined by characteristic prion neuropathology (particularly spongiform degeneration and deposition of pathologic PrP in brain tissue) in the absence of a discernible genetic or infectious cause. Sporadic CJD appears to occur endemically in all populations with an average mortality rate of one to two per million per year, and generally constitutes 85% to 95% of all CJD cases identified through epidemiologic surveillance (5). Genetically caused forms (5% to 15% of classic CJD cases), which apparently also occur universally, are associated with any of over 50 different pathogenic mutations in the host gene that encodes PrP. Some of these genetic forms—in particular, Gerstmann-Sträussler-Scheinker disease and fatal familial insomnia—exhibit distinctive clinicopathologic characteristics and can usually be diagnosed as genetic in origin even when the specific underlying genetic mutation is unknown (6).

Iatrogenic cases of classic CJD (iCJD) have occurred through accidental prion transmission in the health care setting, particularly with therapeutic use of cadaveric tissues or tissue extracts presumably contaminated by donations from individuals with unrecognized sCJD. Although iCJD is very rare, epidemics have been well-documented, with 469 cases reported worldwide as of 2012 (7). In addition, carefully conducted epidemiological studies have yielded new evidence that a fraction of "sporadic" CJD cases may be attributable to remote iatrogenic exposure through surgery, particularly early in life (8,9). Most recently, a new human prion disease, variant CJD (vCJD), emerged in the mid-1990s in the United Kingdom via zoonotic transmission to humans of a prion disease of cattle, bovine spongiform encephalopathy (BSE) (10–12). As of April 2015, 229 cases of vCJD have been reported in residents of 12 countries, including two imported cases in Canada (13,14). This number includes five probable cases, all in the United Kingdom, of secondary (human-to-human) transmission of vCJD infection via the bloodborne route (15–17).

Prompted by the need to better understand the epidemiology of CJD, and the responsibility to promptly and expertly address iatrogenic and zoonotic transmission risks, dedicated systems for prospective, population-based epidemiologic surveillance of CJD began to be established in many countries in the early 1990s (18). In Canada the initial motivator was a question raised by the Krever Commission on the Blood System in Canada, concerning the possibility that CJD might be transmitted by the bloodborne route (19,20). In response, in April 1998 the Government of Canada formally initiated prospective national epidemiologic surveillance of CJD through the Canadian Creutzfeldt-Jakob Disease Surveillance System (CJDSS), now operated by the Public Health Agency of Canada (21). Since 2000, all forms of CJD have been nationally notifiable in Canada, and are now reportable in all provinces and territories. The present article provides an overview of the findings of this national surveillance program over a 16-year period extending from January 1, 1998, to December 31, 2013.

Methods

Data collection

The CJDSS employs an internationally established methodology for epidemiologic surveillance of CJD (18). Following this methodology, after notification by a health professional of a suspected case of CJD in a Canadian resident, CJDSS investigators collaborate to facilitate diagnostic investigations, assemble and organize case files, interpret and disseminate results, support disease reporting, and analyze and manage public health issues. The central case registry and an electronic database are used by the CJDSS to track events, rates and trends of CJD occurrence in Canada. Access to CJDSS investigators for collaborating health professionals, as well as the public, is facilitated by the use of a toll-free telephone line (1-888-489-2999) and a dedicated e-mail address (cjdss@phac-aspc.gc.ca).

Case definitions

Diagnostic investigation of CJD and other subacute encephalopathies in the living patient is frequently challenging (22). Thus, high-quality epidemiologic surveillance entails collection of a variety of types of supporting information, as well as follow-up on individual cases in real time. A diagnosis of *definite CJD* requires neuropathologic examination; thus cranial autopsy is routinely considered. Cases lacking neuropathologic examination can be formally classified as *probable CJD* based on non-neuropathologic criteria, such as clinical profile, results of cerebrospinal fluid (CSF) protein immunoassays, findings of electroencephalography or magnetic resonance imaging, and genetic analysis. Where information is even more limited, it can be feasible to assign the lowest grade formal diagnostic classification of *possible CJD*. Surveillance case definitions employed by the CJDSS, which have been published by the Public Health Agency of Canada's National Notifiable Diseases Surveillance System, are based on those used internationally (18,23).

Mortality rate calculations

Although prospective national surveillance of CJD in Canada was formally initiated as of April 1, 1998, some information was also available on CJD deaths occurring in January, February and March of 1998. Thus, data presented in this report include CJD deaths in Canada recorded by the CJDSS as having occurred between January 1, 1998, and December 31, 2013. With the national scope and population-based approach of the CJDSS, for the purpose of this report the entire population of Canada was used to estimate mortality rates, which are expressed here in units of cases per million population per year. Note also that although no cases of CJD were identified from the Canadian territories (Yukon, Northwest Territories and Nunavut) during the period of surveillance, because the CJDSS was prepared to accept notifications from these regions their populations were included in denominators of national rate estimates. Canadian population and demographic data were adapted from the Statistics Canada CANSIM online socioeconomic database (24). On the basis of these data, over the entire period of study a nominal total of 521,794,297 person-years were included within the scope of surveillance, where a person-year represents membership of an individual in an at-risk population over a period of one year (25).

Exact 95% confidence intervals (CIs) for mortality rate estimates were based on a Poisson model of case counts. The hypothesis of a linear trend in age-adjusted mortality rates over the period of surveillance was tested using nonparametric linear regression, in which the slope of the regression was calculated as the median of slopes connecting all pairs of data points. In addition, a two-tailed test of rank correlation between year and mortality rate was performed using Kendall's tau coefficient, which is based on the proportion of paired observations of two variables in which the respective ranks of the two observations match (26). Standardized mortality ratios (SMRs) for sCJD (defined as the ratio of observed total mortality rates in a study population to "expected" mortality rates in the study population derived using known age-specific rates for a reference population) were estimated for each province. National CJD mortality rates were used to calculate expected rates, again basing 95% CIs on a Poisson model.

With the low numbers of identified cases of genetic prion disease, iCJD and vCJD (see below), only limited statistical analysis was performed for these disease subtypes, with results reported here consisting primarily of case numbers and estimated crude mortality rates for genetic prion disease.

Ethics framework

Formal enrollment of a patient with the CJDSS (*case referral*) is done with written informed consent for each main component of data collection (autopsy, genetics, medical record review and interview) under a protocol approved by the Health Canada–Public Health Agency of Canada Research Ethics Board (Certificate REB-2009-0036). The personal information collected is registered with the Government of Canada as a personal information bank (27).

Results

Diagnostic yield and quality

Between January 1, 1998, and December 31, 2013, the CJDSS opened 1,420 case-referral files on patients suspected of having CJD, or being at risk for CJD (i.e., family members of patients with genetic prion disease). A

total of 3,689 laboratory investigations were performed by Public Health Agency of Canada laboratories, comprising 2,193 CSF protein immunoassays, 635 genetic analyses, and 861 neuropathological examinations including 828 full cranial autopsies (**Table 1**). During the same period the deaths of 662 Canadian residents were attributed to definite or probable CJD, comprising 613 sCJD (92.6%), 43 genetic prion disease (6.5%), 4 iCJD (0.6%), and 2 vCJD (0.3%). Among all 662 CJD diagnoses, 535 (80.8%) were definite and 127 (19.2%) were probable. In addition, 22 deaths were attributed to possible CJD, and 77 cases were unclassifiable. Of the 613 sCJD diagnoses, 498 (81.2%) were definite, and 115 (18.8%) were probable. Among 575 non-CJD diagnoses, CJD was excluded by neuropathology in 308 (53.6%) and by other criteria in 267 (46.4%).

Table 1: Laboratory investigations, case referrals and deaths from CJD in Canada, 1998 to 2013

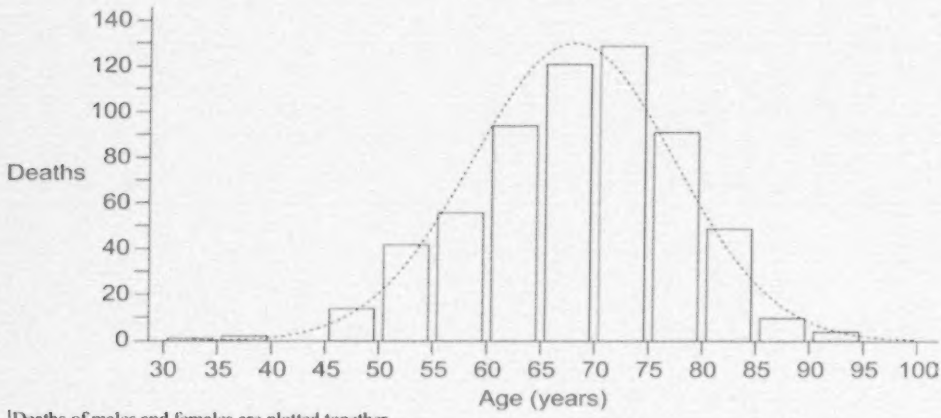
Year[1]	Tests[2]	Referrals[3,4]	Autopsies[5]	Deaths[6]	Definite[7]	Probable[8]
1998	50	44	24	24	20	4
1999	84	63	34	32	28	4
2000	126	82	42	35	32	3
2001	161	101	49	30	23	7
2002	158	103	54	36	32	4
2003	137	75	44	29	27	2
2004	268	90	63	44	33	11
2005	257	97	58	44	37	7
2006	234	80	57	44	37	7
2007	259	101	49	39	33	6
2008	305	100	57	49	34	15
2009	346	104	74	53	46	7
2010	281	76	46	38	31	7
2011	342	102	54	51	40	11
2012	351	103	69	63	46	17
2013	330	99	54	51	36	15
Total	3,689	1,420	828	662	535	127

[1]January 1 to December 31 of each listed year.
[2]All CJD-related laboratory tests, including CSF protein marker analyses, genetic analysis and neuropathologic examination.
[3]*Referral* is defined as enrolment of a patient with the CJDSS.
[4]Note that outcomes of some referrals had not been determined by the end of 2013.
[5]*Autopsy* is defined as full cranial autopsy.
[6]Deaths attributable to definite and probable CJD.
[7]Diagnosis confirmed by neuropathology.
[8]Diagnosis supported by non-neuropathological criteria.

Age and sex distributions for sCJD

Figure 1 illustrates the distribution of ages at death for the 613 sCJD cases. The distribution departed significantly from normality, owing to leftward skewness (D'Agostino-Pearson coefficient –0.297, P = 0.012). Age at death ranged from 33 to 92 years, with a median of 69 [95% CI: 68,70] and inter-quartile range 62,75. Males numbered 291 (47.5%) [95% CI: 43.5,51.4] and females 322 (52.4%) [95% CI: 48.6,56.5], indicating a sex ratio indistinguishable from 1. Age distributions were also very similar between sexes, with males having a median of 68 years [95% CI: 67,70; inter-quartile range: 60,74.75], and females a median of 69 years [95% CI: 67,70; inter-quartile range: 61,75].

Figure 1: Distribution of age at death for 613 cases of sporadic CJD in Canada, 1998 to 2013[1–3]



[1]Deaths of males and females are plotted together.
[2]Ages are grouped into five-year strata.
[3]Normal curve is plotted (dotted line) with mean (67.8) and standard deviation (9.4) of sample.

sCJD mortality

Table 2 provides age-specific case counts and mortality rates for sCJD for five age strata: ≤49 years; 50–59 years; 60–69 years; 70–79 years; and ≥80 years. Crude rates varied widely with age, ranging from 0.05 for ages ≤49 years to 7.11 for ages 70–79 years; only 17 of the 613 sCJD cases (2.8%) were identified in persons ≤49 years of age. Total crude mortality for all age strata over the entire period of surveillance was 1.18, with annual rates ranging from 0.73 in 1998 to 1.78 in 2012. Age-standardized rates were also calculated, using the 2006 Canadian census population as a reference (**Table 2**). These rates ranged from 0.84 for 1998 to 1.65 for 2012, with a total rate over the surveillance period of 1.20. When analyzed by nonparametric linear regression, the age-standardized rates were significantly associated with year, according to the linear function: Rate = 0.029 (Year) – 57.30 [95% CI: 0.002,0.052 for the slope estimate] (**Figure 2**). Kendall's rank-correlation coefficient tau (0.45) was significant at P = 0.017 (two-sided test).

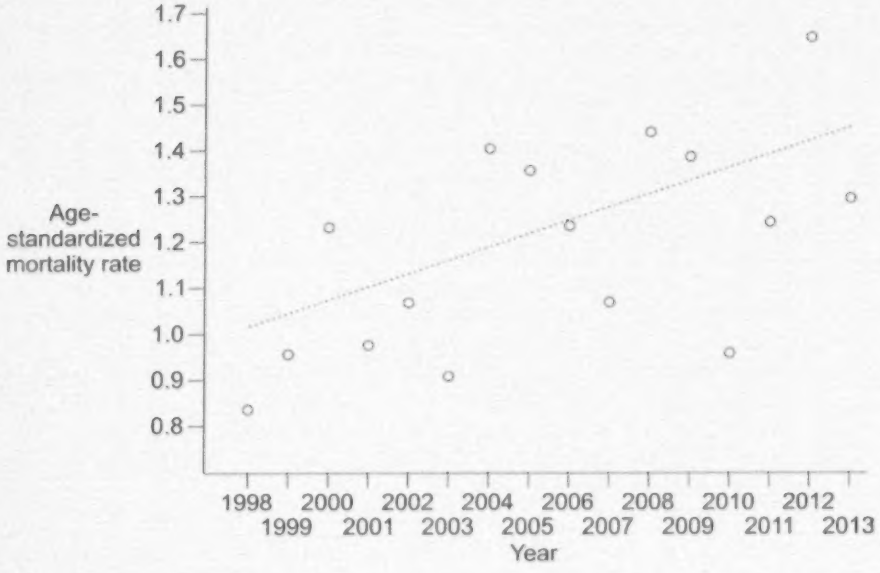
Table 2: Age-specific and total annual mortality from sporadic CJD in Canada, 1998 to 2013

Year[1]	Age[2]						Mortality[3]	
	≤49	50–59	60–69	70–79	≥80	All ages	Crude[4]	Age-adjusted[5]
1998	0	3	5	12	2	22	0.73 [0.46,1.10]	0.84 [0.52,1.27]
1999	2	4	9	10	2	27	0.89 [0.58,1.29]	0.96 [0.63,1.39]
2000	1	4	5	14	8	32	1.04 [0.71,1.47]	1.23 [0.84,1.75]
2001	0	4	12	8	3	27	0.87 [0.57,1.26]	0.98 [0.64,1.42]
2002	2	5	6	14	4	31	0.99 [0.67,1.40]	1.07 [0.73,1.52]
2003	2	1	13	11	0	27	0.85 [0.56,1.24]	0.91 [0.60,1.32]
2004	0	10	14	13	5	42	1.31 [0.95,1.77]	1.40 [1.01,1.90]
2005	1	8	12	21	0	42	1.30 [0.94,1.76]	1.36 [0.98,1.83]
2006	0	5	13	17	4	39	1.19 [0.85,1.63]	1.24 [0.88,1.69]
2007	2	7	14	8	4	35	1.06 [0.74,1.48]	1.07 [0.75,1.49]
2008	0	9	13	18	8	48	1.44 [1.06,1.91]	1.44 [1.06,1.91]

2009	1	7	19	16	5	48	1.42 [1.05,1.89]	1.39 [1.02,1.84]
2010	2	6	19	4	4	35	1.03 [0.71,1.43]	0.96 [0.67,1.33]
2011	1	8	20	12	5	46	1.33 [0.98,1.78]	1.24 [0.91,1.66]
2012	1	13	22	20	6	62	1.78 [1.36,2.28]	1.65 [1.26,2.11]
2013	2	4	19	22	3	50	1.42 [1.05,1.87]	1.30 [0.96,1.71]
All years	17	98	215	220	63	613	1.18 [1.08,1.27]	1.20 [1.11,1.30]
Mortality[3]	0.05 [0.03,0.08]	1.41 [1.14,1.72]	4.59 [4.00,5.25]	7.11 [6.20,8.11]	3.44 [2.64,4.40]	1.18 [1.08,1.27]	–	–

[1]Time intervals are from January 1 to December 31 for each calendar year.
[2]Age is expressed as number of complete years of life at time of death.
[3]Mortality rates are expressed in deaths per million population per year, with Poisson-based 95% confidence interval (in parentheses).
[4]Based on Statistics Canada estimates of total Q4 Canadian population per year.
[5]Based on 2006 Canadian census.

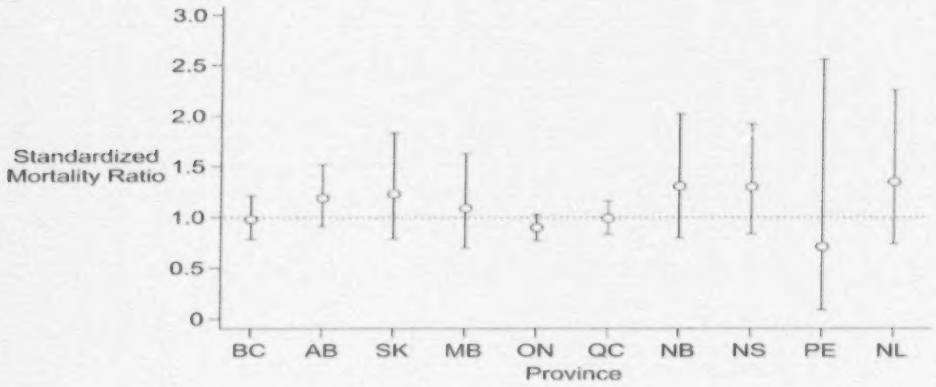
Figure 2: Age-standardized mortality from sporadic CJD in Canada, 1998 to 2013[1–3]



[1]Mortality rates are expressed per million population per year.
[2]Direct standardization used 2006 Canadian census population as reference population.
[3]Slope of nonparametric linear regression (dotted line) = median of slopes between all data pairs.

To examine the possibility that CJD mortality rates varied geographically during the study period, SMRs and 95% CIs were estimated for each province, using the estimated national age-specific mortality rates as a reference to calculate expected rates. The results are shown in **Figure 3**. It can be seen that both the SMR estimates and the widths of their respective 95% CIs varied considerably, with the latter expected given differing case numbers among provinces with different population sizes [range: <5 cases (PEI) to 209 cases (Ontario)]. Despite this variation, the 95% CIs of SMRs for all provinces included the value 1.0, indicating a lack of statistical support for geographic variation in mortality rates.

Figure 3: Standardized mortality ratios for sporadic CJD in Canadian provinces, 1998 to 2013[1-4]



[1]Abbreviations of province names: BC—British Columbia; AB—Alberta; SK—Saskatchewan; MB—Manitoba; ON—Ontario; QC—Québec; NB—New Brunswick; NS—Nova Scotia; PE—Prince Edward Island; NL—Newfoundland and Labrador
[2]Open symbols indicate point estimates of standardized mortality ratios (SMRs).
[3]Vertical bars indicate 95% confidence intervals for SMRs, based on Poisson model.
[4]SMRs were calculated using national average sCJD mortality rate as the reference rate.

Genetic prion disease, iCJD and vCJD

Basic features of the 43 identified cases of genetic prion disease are summarized in **Table 3**. Cases of all three known classic clinicopathologic presentations of genetic prion disease (CJD, Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia) were identified. Autopsy rates were high in this group as well, with 33 of the 43 diagnoses (77%) supported by neuropathology in addition to an identified mutation in the host gene that encodes PrP. Interestingly, 9 of the 20 cases of Gerstmann-Sträussler-Scheinker disease (45%) were only discovered at autopsy and lacked an identified mutation because genetic analysis had not been requested. Clearly the total mortality rate from genetic prion diseases over the study period was much lower than that for sCJD, at 0.08 per million per year [95% CI: 0.06,0.11]. Lastly, four cases of iCJD (all with receipt of a *dura mater* graft as a risk factor) and two cases of vCJD (one definite and one probable) were identified.

Table 3: Characteristics of 43 cases of genetic prion disease identified in Canada, 1998 to 2013

Subtype[1]	Mutation identified[2]	Mutation not identified[3]	Definite (%)[4]	Probable (%)[5]	Total
CJD	21	0	16 (76)	5 (24)	21
GSS	11	9	16 (80)	4 (20)	20
FFI	2	0	1 (50)	1 (50)	2
All	34	9	33 (79)	10 (21)	43

[1]CJD—Creutzfeldt-Jakob disease; GSS—Gerstmann-Sträussler-Scheinker disease; FFI—fatal familial insomnia.
[2]Causative DNA change identified.
[3]Causative DNA change not identified (no genetic analysis performed).
[4]Diagnosis confirmed by neuropathology.
[5]Diagnosis supported by non-neuropathological criteria.

Discussion

Based on national prospective epidemiologic surveillance, a total of 662 deaths were attributed to all forms of CJD in Canada between 1998 and 2013. For the most common etiologic subtype, sCJD (92.6% of all cases), we estimated an overall average crude mortality rate of 1.18 definite and probable deaths per million per year. We also confirmed the strong relationship between crude sCJD mortality rates and age, with a very low age-specific rate of 0.05 in those under 50 years, and a maximum of 7.11 in the age stratum 70 to 79 years. Another known epidemiologic pattern confirmed by our data was the lack of detectable sex bias in mortality rates. Evidence was found for a modest net upward trend in age-standardized sCJD mortality rate over the 16-year period. However,

consistent with strong national coverage, no statistical support was found for geographic deviation in rate with respect to that expected from the national rate for any of the 10 Canadian provinces in which cases were observed.

The epidemiology of CJD has been studied systematically for nearly four decades (5–7,12,28–30). Early estimates of mortality based primarily on retrospective approaches to case identification typically fell well below one per million per year (29). A more structured, prospective approach to case finding undertaken in France between 1978 and 1982 provided the first well-controlled demonstration of a within-country gradient of observed rates (0.56 for France, 0.86 for metropolitan Paris, and 1.19 for the city of Paris), representing a positive correlation of rates with degree of urbanization and suggesting a significant ascertainment effect (30). The combination of retrospective and prospective studies in France and later in the United Kingdom demonstrated a rise in apparent mortality with time as well, which was also attributed to improved case ascertainment (30,31).

More recent estimates of CJD mortality rates have tended toward the range of one to two per million per year (5). This has occurred in parallel with methodological enhancements such as establishment of national registries, application of a prospective approach, formulation of standard case definitions, recognition of disease subtypes, development and validation of diagnostic tools, and increased awareness among collaborating health professionals, all strengthening the plausibility of improved ascertainment, particularly in older patients and/or patients with less typical presentations (5,32). In Switzerland, a temporary increase in CJD mortality in the years 2001 to 2004 was ultimately attributed to heightened awareness of the disease as a result of the vCJD epidemic in the United Kingdom; the authors went so far as to suggest that the "true" mortality rate for sCJD may exceed two per million per year (33). In a recent study incorporating data from 10 countries, significant statistical correlations were demonstrated between observed sCJD mortality rate and several indices of surveillance intensity, including rates of case notification to the central registry as well as rates of autopsy and other laboratory investigations (34). In light of these considerations, we believe that the observed upward trend in age-standardized sCJD mortality rates in Canada between 1998 and 2013 can be best accounted for by gradually improving case ascertainment, rather than extrinsic causes leading to a real rate elevation. Thus, although under-ascertainment could legitimately be considered a potential limitation of the present study, such a positive trend suggests that further improvements could be realized. Given the strong correlation of mortality rates with age, it is also worth keeping in mind the possibility that population aging will contribute to real increases in sCJD rates in the future.

The surveillance findings presented here confirm and extend those of earlier published studies that included either retrospective or prospective data on CJD mortality in Canada, and are consistent with those of other CJD surveillance systems internationally (5,34–36). In addition to providing high-quality estimates of overall, age-specific and sex-specific mortality rates for classic forms of CJD, the Canadian CJD Surveillance System has demonstrated an ability to detect, investigate and deal with rare events, such as four identified cases of iCJD and two of vCJD. Our results indicate, therefore, that a collaborative environment has been established for robust, national prospective epidemiologic surveillance of CJD in Canada.

It is important for health professionals to remain alert to the possibility of CJD in patients. Likewise, it is important for public health professionals to use the surveillance data and the resulting epidemiologic insights to assess and mitigate any public health risks associated with the ongoing, possibly under-ascertained and potentially increasing occurrence of classic forms of CJD. Finally, there are persisting uncertainties regarding the epidemiology of vCJD and possible new zoonotic prion diseases, which warrant continued vigilance for novel CJD events or trends (37,38).

Acknowledgements

The authors express their sincere gratitude to the many health professionals, health care institutions, service providers and, above all, affected patients and families, without whose generous collaboration, this work would not have been possible. Special thanks are also extended to the staff of the Prion Laboratory Section, National Public Health Laboratories, Public Health Agency of Canada, and of the Neuropathology Laboratory Services of the University of Ottawa for their skill and dedication in delivering high-quality laboratory diagnostic services. The Canadian population and demographic information used in this report was adapted from Statistics Canada; this does not constitute an endorsement by Statistics Canada of this publication.

Conflict of interest

None.

Funding

Funding for the Canadian CJD Surveillance System is provided through the Public Health Agency of Canada's Prion Diseases Program.

References

(1) Prusiner SB. Prions. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13363–83.

(2) Kraus A, Groveman BR, Caughey B. Prions and the potential transmissibility of protein misfolding diseases. Annu Rev Microbiol. 2013;67:543–64.

(3) Cashman NR. Propagated protein misfolding: New opportunities for therapeutics, new public health risk. CCDR. 2015;41(8): 196-200

(4) Belay ED, Schonberger LB. The public health impact of prion diseases. Ann Rev Public Health. 2005;26:191–212.

(5) Ladogana A, Puopolo M, Croes EA, Budka H, Jarius C, Collins S, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology. 2005 May 10;64(9):1586–91.

(6) Kovacs GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, van Duijn C, et al. Genetic prion disease: the EUROCJD experience. Hum Genet. 2005 Nov;118(2):166–74.

(7) Brown P, Brandel JP, Sato T, Nakamura Y, MacKenzie J, Will RG, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis. 2012 Jun;18(6):901–7.

(8) de Pedro-Cuesta J, Mahillo-Fernandez I, Rabano A, Calero M, Cruz M, Siden A, et al. Nosocomial transmission of sporadic Creutzfeldt-Jakob disease: Results from a risk-based assessment of surgical interventions. J Neurol Neurosurg Psychiatry. 2011 Feb;82(2):204–12.

(9) de Pedro-Cuesta J, Mahillo-Fernandez I, Calero M, Rabano A, Cruz M, Siden A, et al. Towards an age-dependent transmission model of acquired and sporadic Creutzfeldt-Jakob disease PLoS One. 2014 Oct 3;9(10):e109412.

(10) Coulthart MB, Cashman NR. Variant Creutzfeldt-Jakob disease: A summary of current scientific knowledge in relation to public health. CMAJ. 2001 Jul 10;165(1):51–8.

(11) Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, et al. A new variant of Creutzfeldt-Jakob disease in the UK Lancet. 1996 Apr 6;347(9006):921–5.

(12) Diack AB, Head MW, McCutcheon S, Boyle A, Knight R, Ironside JW, et al. Variant CJD. 18 years of research and surveillance. Prion. 2014;8(4):286–95.

(13) Jansen GH, Voll CL, Robinson CA, Gervais R, Sutcliffe T, Bergeron C, et al. First case of variant Creutzfeldt-Jakob disease in Canada. CCDR. 2003 Jul 1;29(13):117–20.

(14) Public Health Agency of Canada (PHAC). Variant Creutzfeldt-Jakob disease in a Canadian resident. CCDR Weekly. Inf Dis News Brief. 2011;4(10).

(15) Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang. 2006 Oct;91(3):221–30.

(16) Brown P. Creutzfeldt-Jakob disease: Reflections on the risk from blood product therapy. Haemophilia. 2007 Dec;13 Suppl 5:33–40.

(17) Peden A, McCardle L, Head MW, Love S, Ward HJ, Cousens SN, et al. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. Haemophilia. 2010 Mar;16(2):296–304.

(18) World Health Organization (WHO). WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease. Geneva: WHO; 2003. http://whqlibdoc.who.int/publications/2003/9241545887.pdf

(19) Krever H. Commission of Inquiry on the Blood System in Canada: Final Report. Ottawa: Government of Canada; 1997.

(20) Ricketts MN, Cashman NR, Stratton EE, ElSaadany S. Is Creutzfeldt-Jakob disease transmitted in blood? Emerg Infect Dis. 1997 Apr–Jun;3(2):155–63.

(21) Coulthart MB, Stratton EE, Ricketts MN. Surveillance of Creutzfeldt-Jakob disease in Canada. Can J Infect Dis. 1997;8(5):241–4.

(22) Murray K. Creutzfeldt-Jakob disease mimics, or how to sort out the subacute encephalopathy patient. Pract Neurol. 2011 Feb;11(1):19–28.

(23) Public Health Agency of Canada. Case definitions for communicable diseases under national surveillance: 2009. CCDR. 2009;35(S2):1–128.

(24) CANSIM: Canadian Socioeconomic Information Management database, Table 051-0001 (reference date 2014-09-24), Table 97-551-X2006008 (reference date 2007-07-17). www.statcan.gc.ca

(25) Porta M. A Dictionary of Epidemiology. 4th ed. New York: Oxford University Press; 2008.

(26) Conover WJ. Practical Nonparametric Statistics. 3rd ed. New York: Wiley; 1999.

(27) Info Source: Sources of federal government and employee information. Record number PHAC 008 180, Treasury Board Secretariat registration number 009940, Bank number PHAC PPU 286. 2014 Sep 3. http://www.phac-aspc.gc.ca/about_apropos/atip-aiprp/info-source-eng.php

(28) Masters CL, Harris JO, Gajdusek DC, Gibbs CJ, Jr, Bernoulli C, Asher DM. Creutzfeldt-Jakob disease: Patterns of worldwide occurrence and the significance of familial and sporadic clustering. Ann Neurol. 1979 Feb;5(2):177–88.

(29) Will RG, Matthews WB, Smith PG, Hudson C. A retrospective study of Creutzfeldt-Jakob disease in England and Wales 1970–1979. II: Epidemiology. J Neurol Neurosurg Psychiatry. 1986 Jul;49(7):749–55.

(30) Brown P, Cathala F, Raubertas RF, Gajdusek DC, Castaigne P. The epidemiology of Creutzfeldt-Jakob disease: Conclusion of a 15-year investigation in France and review of the world literature. Neurology. 1987 Jun;37(6):895–904.

(31) Cousens SN, Zeidler M, Esmonde TF, De Silva R, Wilesmith JW, Smith PG, et al. Sporadic Creutzfeldt-Jakob disease in the United Kingdom: Analysis of epidemiological surveillance data for 1970–96. BMJ. 1997 Aug 16;315(7105):389–95.

(32) Stoeck K, Hess K, Amsler L, Eckert T, Zimmermann D, Aguzzi A, et al. Heightened incidence of sporadic Creutzfeldt-Jakob disease is associated with a shift in clinicopathological profiles. J Neurol. 2008 Oct;255(10):1464–72.

(33) Ruegger J, Stoeck K, Amsler L, Blaettler T, Zwahlen M, Aguzzi A, et al. A case-control study of sporadic Creutzfeldt-Jakob disease in Switzerland: Analysis of potential risk factors with regard to an increased CJD incidence in the years 2001–2004. BMC Public Health. 2009 Jan 14;9:18. doi: 10.1186/1471-2458-9-18.

(34) Klug GM, Wand H, Simpson M, Boyd A, Law M, Masters CL, et al. Intensity of human prion disease surveillance predicts observed disease incidence. J Neurol Neurosurg Psychiatry. 2013 Dec;84(12):1372–7.

(35) Stratton E, Ricketts MN, Gully PR. The epidemiology of Creutzfeldt-Jakob disease in Canada: A review of mortality data. Emerg Infect Dis. 1997 Jan–Mar;3(1):63–4.

(36) Elsaadany S, Semenciw R, Ricketts M, Mao Y, Giulivi A. Epidemiological study of Creutzfeldt-Jakob disease death certificates in Canada, 1979–2001. Neuroepidemiology. 2005;24(1–2):15–21.

(37) Saunders SE, Bartelt-Hunt SL, Bartz JC. Occurrence, transmission, and zoonotic potential of chronic wasting disease. Emerg Infect Dis. 2012 Mar;18(3):369–76.

(38) Gill ON, Spencer Y, Richard-Loendt A, Kelly C, Dabaghian R, Boyes L, et al. Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: Large scale survey. BMJ. 2013 Oct 15;347:f5675.

Innovation

A new diagnostic test for Creutzfeldt-Jakob disease: Real-time quaking-induced conversion (RT-QuIC)

Godal D[1], Simon SLR[1], Cheng K[1,2], Knox JD[1,3*]

[1]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB
[2]Department of Anatomy and Cell Sciences, University of Manitoba, Winnipeg, MB
[3]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB

*Correspondence: david.knox@phac-aspc.gc.ca

Abstract

Currently, definitive diagnoses of Creutzfeldt-Jakob disease (CJD) require an examination of the brains of deceased patients. In cooperation with an international consortium, Canada's National Microbiology Laboratory is performing a validation of a new test that can diagnose CJD in living patients. This test, the real-time quaking-induced conversion (RT-QuIC) assay, directly detects the disease associated form of the prion protein in cerebrospinal fluid. If validated, RT-QuIC will represent a major advance in the diagnoses of patients suspected to have CJD.

Introduction

Creutzfeldt-Jakob disease (CJD) is a rare, untreatable, uniformly fatal brain disorder. The disease most commonly affects older adults at a rate of one case of CJD diagnosed per million people each year (1). The first signs often include anxiety, confusion and memory loss resembling those of other neurological disorders such as Alzheimer's disease and Huntington's disease. There are several subtypes of CJD and the signs of disease progression are highly variable. However, it is common to observe difficulties with balance, hand coordination, walking, vision and swallowing, as well as involuntary jerky movements (2). Ultimately, people lose their ability to move and speak; death is often caused by pneumonia or other secondary infections.

These fatal neurological diseases are caused by novel transmissible agents called prions that are not conventional viruses or bacteria but rather are composed of an abnormal form of a host protein. This key biological fact precludes the use of technologies currently applied to the direct detection of other infectious agents. Presently, definitive diagnoses of CJD require an examination of the brains of deceased patients revealing prion protein deposits and associated nerve cell loss resulting in the brain having a sponge-like appearance.

The objective of this article is to summarize three different types of CJD, to describe a new method recently developed to diagnose CJD, and to consider the potential implications for patients and clinical/laboratory practice.

Classification of CJD

Creutzfeldt-Jakob disease can be classified into three broad categories: sporadic CJD, genetic CJD and acquired CJD. Sporadic CJD (sCJD) represents up to 85% of observed cases, develops for no apparent reason and usually affects people in their 60s. Depression is a common early symptom and most people with sCJD die within six months of diagnosis.

Genetic CJD (gCJD) can present like sporadic CJD or may present as a slowly progressing dementia that develops over years. Some of the more common CJD mutations have been tracked through large multigenerational families and display autosomal dominant inheritance. Nonetheless, in 50% of genetic CJD diagnoses there is no familial history of CJD or other neurological disorders suggesting low penetrance (3).

Acquired CJD, accounting for less than 1% of cases, results from exposure to the infectious agent. Iatrogenic CJD describes cases of acquired CJD related to medical procedures. New variant CJD (vCJD) is acquired through dietary exposure to beef infected with bovine spongiform encephalopathy (BSE), the scientific term for mad cow disease. Due to the effective implementation of public health measures (4), the risk of acquiring variant CJD in Canada is nearly nonexistent. Indeed, there has never been a case of domestically acquired variant CJD reported in Canada (1).

Current practice

Pre-mortem diagnoses of any form of CJD rely on clinical presentation, characteristic electroencephalogram (EEG) and magnetic resonance imaging (MRI) patterns. The appearance of surrogate biomarkers, typically a change in the amount of specific proteins in cerebrospinal fluid (CSF), provides additional supporting diagnostic data.

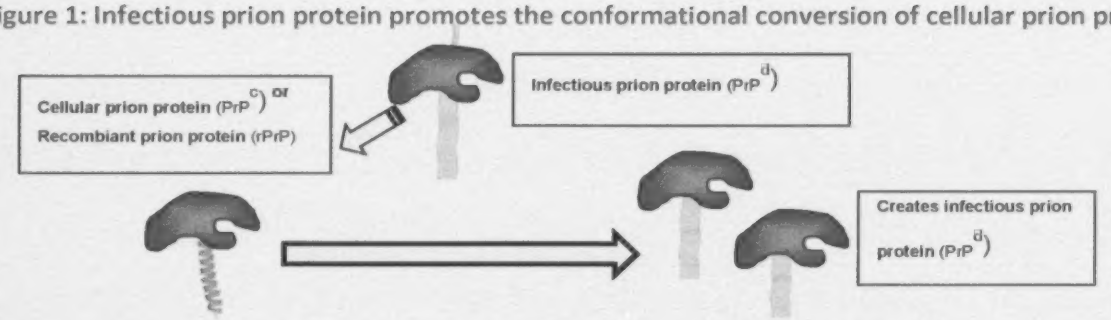
Currently, the Prion Laboratory Section at the National Microbiology Laboratory measures the level of three proteins (14-3-3, tau and S100) in CSF, to support clinicians dealing with suspected cases of CJD. Increased levels of these three surrogate markers are consistent with a diagnosis of CJD, but they have variable sensitivity and specificity and consequently cannot be used to definitively diagnose CJD (5).

The molecular basis of the new test

At the molecular level CJD disease progression is characterized by the conversion of the normally expressed cellular prion protein, PrP^{c}, into the misfolded disease associated isoform, PrP^{d} (**Figure 1**). The infectious form of the prion protein, PrP^{d}, acts as a "seed" and interacts with normal cellular form of the prion protein, PrP^{c}, and promotes its conversion to the infectious form. As the disease progresses the amount of PrP^{d} available to convert PrP^{c} increases, accelerating the conversion of PrP^{c} to PrP^{d}. Eventually this leads to the generation of the large amounts of PrP^{d} observed in the brains of CJD patients post-mortem.

In contrast to what is observed in the brain, only low amounts of PrP^{d} are present in other tissues making detection in an easily accessible sample problematic. A solution to this challenge has been the development of a technique, known as real-time quaking-induced conversion (RT-QuIC). RT-QuIC exploits the ability of PrP^{d} to promote the conformational conversion of PrP^{c} (6-8). In RT-QuIC a recombinant prion protein (rPrP) made in the laboratory is used as the substrate and patient CSF samples, potentially containing PrP^{d}, are tested to see if they will "seed" the conversion process (**Figure 1**). Cycles of shaking and incubation will cause infected CSF, containing PrP^{d}, to generate greatly increased amounts of $rPrP^{d}$ facilitating subsequent detection by traditional methods.

Figure 1: Infectious prion protein promotes the conformational conversion of cellular prion protein


Cellular prion protein (PrP^c) or
Recombiant prion protein (rPrP)
Infectious prion protein (PrP^d)
Creates infectious prion protein (PrP^d)

Legend: In RT-QuIC the PrP^d present in infected samples interacts with laboratory produced rPrP causing a conformational conversion that can be monitored in real time.

Summary of test results

RT-QuIC has been demonstrated to be a highly sensitive and specific method to detect the presence of the disease-associated isoform of the prion protein, PrP^d, in the CSF of patients with sporadic CJD (9-13). Direct detection of the disease-causing agent is a major advance in the development of a diagnostic test for sporadic CJD as it can be used for definitive diagnoses while the patient is still alive. However, ongoing validation studies have demonstrated that the outcomes of RT-QuIC reactions depend on the exact conditions employed (biochemical, chemical and physical), as well as the nature of the sample and recombinant substrate. The protocol under evaluation by the National Microbiology Laboratory has been tested extensively for the detection of sporadic CJD (10,11,15,16). Using this protocol the Prion Laboratory Section achieved 100% sensitivity and 100% specificity in the evaluation of an external sporadic CJD infected CSF proficiency panel. This is comparable to the 91% sensitivity and 98% specificity reported by other laboratories (8,9). Being part of an international consortium using the same standardized protocol should permit analyses of sufficient numbers of the rarer vCJD and gCJD samples to determine the efficacy of our version of RT-QuIC in these cases.

Discussion

The establishment of a specific and sensitive test will aid health care workers as well as patients and their families in dealing with suspected cases of CJD. A more specific and sensitive diagnostic test for CJD will also help address wider public health considerations concerning the transmission of CJD from patient to patient through the use of contaminated surgical instruments and tissues, including blood. Once validation is completed RT-QuIC will be a service offered by the Prion Laboratory Section of the National Microbiology Laboratory, making a more sensitive and specific ante-mortem test for CJD available to Canadian health care professionals.

Acknowledgements

We acknowledge with thanks the members of the EU Joint Programme–Neurodegenerative Disease Research (JPND) Consortium for the provision of samples and expertise, and the Bristol Institute for Transfusion Sciences (BITS) for the provision of rPrP.

Conflict of interest

None

Funding

This work was supported by the Public Health Agency of Canada.

References

(1) Coulthart MB, Jansen GH, Connolly T, D'Amour R, Kruse J, Lynch J et al. Creutzfeldt-Jakob disease mortality in Canada, 1998–2013 Can Comm Dis Rep 2015;41:182-191.

(2) Rosenbloom MH, Atri A. The evaluation of rapidly progressive dementia. Neurologist. 2011;17(2):67–74.

(3) Kovács GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, van Duijn C, Collins SJ, Boyd A, Giulivi A, Coulthart M, Delasnerie-Laupretre N, Brandel JP, Zerr I, Kretzschmar HA, de Pedro-Cuesta J, Calero-Lara M, Glatzel M, Aguzzi A, Bishop M, Knight R, Belay G, Will R, Mitrova E. Genetic prion disease: The EUROCJD experience. Hum Genet. 2005 Nov;118(2):166–74.

(4) Canadian Food Inspection Agency. Evaluation of Bovine Spongiform Encephalopathy (BSE) Management Program. http://www.inspection.gc.ca/about-the-cfia/accountability/other-activities/audits-reviews-and-evaluations/evaluation-of-the-bse-management-program/evaluation/eng/1419265462091/1419265463216

(5) Coulthart MB, Jansen GH, Olsen E, Godal DL, Connolly T, Choi BC, Wang Z, Cashman NR. Diagnostic accuracy of cerebrospinal fluid protein markers for sporadic Creutzfeldt-Jakob disease in Canada: A 6-year prospective study. BMC Neurol. 2011 Oct 27;11:133. doi: 10.1186/1471-2377-11-133.

(6) Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature. 2001;411(6839):810–3.

(7) Atarashi R, Moore RA, Sim VL, Hughson AB, Dorward D, Onwubiko HA, Priola SA, Caughey B. Ulrtasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. Nat Methods. 2007;4(8):645–50.

(8) Wilham JM, Orrú CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. PLoS Pathog. 2010;Dec 2;6(12):e1001217.

(9) Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, Yamada M, Mizusawa H, Kitamoto T, Klug G, McGlade A, Collins SJ, Nishida N. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nat. Med. 2011;17:175–8.

(10) Peden AH, McGuire LI, Appleford NEJ, Mallinson G, Wilham JM, Orrú CD ,Caughey B, Ironside JW, Knight RS, Will RG, Green AJE, Head MW. Sensitive and specific detection of sporadic Creutzfeldt–Jakob disease brain prion protein using real-time quaking-induced conversion. J. Gen Virol. 2012:93:438–49.

(11) McGuire LI, Peden AH, Orrú CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, Knight RS, Green AJ. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol. 2012:72:278–85.

(12) Cramm M, Schmitz M, Karch A, Zafar S, Varges D, Mitrova E, Schroeder B, Raeber A, Kuhn F, Zerr I. Characteristic CSF prion seeding efficiency in humans with prion diseases. Mol Neurobiol. 2015 Feb;51(1):396–405. doi: 10.1007/s12035-014-8709-6.

(13) Orrú CD, Bongianni M, Tonoli G, Ferrari S, Hughson AG, Groveman BR, Fiorini M, Pocchiari M, Monaco S, Caughey B, Zanusso G. A test for Creutzfeldt-Jakob disease using nasal brushings. N Engl J Med. 2014;371:519–29.

(14) Cheng K, Sloan A, Avery KM, Coulthart M, Carpenter M, Knox JD. Exploring physical and chemical factors influencing the properties of recombinant prion protein and the real-time quaking-induced conversion (RT-QuIC) Assay. PloS One. 2014 Jan3;9:1. doi: 10.1371/journal.pone.0084812.

(15) Orrú CD, Wilham IM. Raymond LD, Kuhn F, Schroeder B, Raiber AI, Caughey B. Prion disease blood test using immunoprecipitation and improved quaking-induced conversion. mBio. 2011;2e00078-11.

(16) Sano K, Satoh K, Atarashi R, Takashima H, Iwasaki Y, Yoshida M, Sanjo N, Murai H, Mizusawa H, Schmitz M, Zerr I, Kim YS, Nishida N. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. PLoS One. 2013; 8(1):e54915.

Commentary

Propagated protein misfolding: New opportunities for therapeutics, new public health risk

Cashman NR[1]*

[1]Affiliation: Faculty of Medicine, University of British Columbia, Vancouver BC

*Correspondence: neil.cashman@vch.ca

Abstract

There is now good consensus that propagated protein misfolding is the underlying mechanism for the infectious prion diseases (Creutzfeldt-Jakob disease in humans, scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, and chronic wasting disease in deer and elk). Over the past decade it has become increasingly clear that other diseases, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis may progress via the same mechanism, involving a disease-specific polypeptide rather than the prion protein. Recent literature in these non-prion neurodegenerative diseases also points to the existence of multiple "strains" that express themselves differently in different contexts, resulting in different disease phenotypes. The probable cause of these neurodegenerative diseases is now referred to collectively as "propagated protein misfolding." Propagated protein misfolding raises many opportunities for new therapeutics and diagnostics. However, it also raises the theoretical risk of iatrogenic transmission, although experimental support for this notion is limited at present.

Introduction

The hypothesis that a misfolded protein could confer its misfold on a neighbouring normal protein and cause disease was widely regarded as heretical when first proposed in 1982 by Stanley Prusiner (1), but this idea is now well-accepted, and was sanctioned by a Nobel Prize in 1997. There is now good consensus that propagated protein misfolding of host prion protein (encoded in humans by the gene PRNP) is the underlying mechanism for the infectious prion diseases (Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease in deer and elk). What has become apparent more recently is the notion that misfolded proteins may be part of the underlying pathology in more common neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, and possibly some non-neurological conditions such as type II diabetes mellitus and peripheral amyloidosis (2).

The objective of this article is to provide some highlights of how non-prion protein misfolding is mediated through polypeptides in neurodegenerative diseases, to explore some of the opportunities this creates for both diagnosis and therapy, and to identify the theoretical risk this poses for iatrogenic transmission. See text box for common protein misfolding terminology.

Protein misfolding terminology

Epitope—for proteins, a region or sequence of amino acids which can be recognized by a specific antibody.

Exosomes—small (~100nM) membrane-bound vesicles which are secreted by living cells, containing protein and nucleic acids.

Prion—infectious aggregate of prion protein, responsible for transmission of CJD[1], scrapie, BSE[2] and chronic wasting disease.

Propagated protein misfolding—prion-like transmission of protein misfolding between cells in an organ such as the brain, mediated by protein aggregates and/or exosomes. Although prion-like, no evidence to date shows natural transmission of disease among or between species.

PPM[3] seeding—application of prion aggregation theory to propagated protein misfolding aggregates, requiring a specific event in which misfolded monomers aggregate into a productive template (slow kinetics) for recruitment of additional monomers (rapid kinetics).

PPM[3] strains—application of prion strain behaviour to non-prion propagated protein misfolding, correlating with differences in propagated structure of aggregates of implicated proteins.

Post-translational covalent modification—any change in a protein mediated by a chemical bond, such as oxidation and glycosylation.

[1]CJD = Creutzfeldt-Jakob disease [2]BSE = bovine spongiform encephalopathy [3]PPM = propagated protein misfolding

Misfolding prions and other polypeptides

We now know that propagated protein misfolding can occur either through infectious prions, composed of aggregated misfolded host prion protein, or through the propagated aggregation of other proteins implicated in neurodegenerative diseases (no natural infectious lifecycle). There is recent experimental proof, for example, that the following propagated misfolded proteins are now associated with the following neurodegenerative disorders: Amyloid-beta (Abeta) oligomers/fibrils in Alzheimer's disease (3); alpha-synuclein in Parkinson's disease and Lewy body dementia (4) and multiple systems atrophy (5); superoxide dismutase 1 (SOD1) (6) and transactive response DNA binding protein 43 (TDP43) (7) in amyotrophic lateral sclerosis, and tau in the tauopathies, as well as Alzheimer's disease (8). Both prion infection and non-prion neurodegenerative diseases progress through propagated protein misfolding.

Different propagated protein misfolding strains may drive different disease progression

Infectious prions (CJD, scrapie, BSE and chronic wasting disease) do not exist in one monolithic form, but rather can exhibit "strain behaviour." In sheep scrapie, for example, when the first infectious prions were transmitted to mouse models, approximately 20 strains were cloned—defined on the basis of incubation time, brain regions predominantly affected, and biochemical features such as glycosylation preferences. Very recently, it has been shown that non-infectious prion-like propagated protein misfolding agents causing neurodegenerative diseases also exhibit strain behaviour. In studies of Alzheimer's disease, strain properties of aggregated Abeta in human brain have been found to correlate with rate of progression of disease (9,10). In Parkinson's disease and in multiple systems atrophy, different strains of aggregated alpha synuclein have been identified in vitro and in vivo (11). In amyotrophic lateral sclerosis, the propagated misfolding of SOD1 has been shown to display at least two distinct forms in a mouse model of the disease (12). And tauopathy has been found to propagate in multiple strains (13) that correspond to the clinical features and rate of progression in human disease.

New therapeutics and diagnostics for protein misfolding diseases

A new drug development mindset is required for protein misfolding diseases. For over a century, pharmacological science has been highly successful in applying small molecules to fixed structures: this has been an effective strategy for selective targeting of channels, pores and enzyme surface pockets and pits that can induce change in protein activity. However, the small molecule approach does not generally apply in protein

misfolding where the targets are often large and unstable. Moreover, propagated protein misfolding diseases involve protein–protein interactions which have proven to be very difficult to target with drugs.
The identification of strains provides opportunity for "precision medicine" treatment of neurodegenerative diseases. But there is a downside: data have accumulated indicating that treatment of one strain of infectious prions can "select" for another strain that can be more pathogenic. With this precedent from infectious prions, we should be aware that blocking propagated protein misfolding in other neurodegenerative diseases could also select for emergent strains that will require their own specific treatment.

Our own work has envisioned that effective treatment and diagnosis of protein misfolding diseases will require a new paradigm based on the rational identification of selective epitopes (or antibody targets) in the misfolded proteins that are key to the disease process. Selective antibody targeting of misfolded proteins is effective by several mechanisms, including the neutralization of aggregate cytotoxicity and inhibition of prion-like propagated protein misfolding (14).

In some conditions, such as infectious prion disease and Alzheimer's disease, the specific targeting of misfolded propagating proteins is like finding "a needle in a haystack" where the misfolded species may be present in a thousand-fold to a million-fold lower concentration than the natively folded species—a situation we have dubbed "target distraction." But the advantage of specific immunotherapy for misfolded proteins is that it would spare normally folded protein isoforms from autoimmune recognition.

Misfolding-specific epitopes can be produced by gain or loss of structure

Misfolding epitopes for immunotherapy or disease biomarkers can appear by two mechanisms in protein misfolding diseases: a gain or loss of structure not present in normal protein isoforms. Gain of structure can occur through generation of neoepitopes associated with post-translational covalent modification (mediated by the formation of a chemical bond). Non-covalent forces can also engender neoepitopes. For example, we have identified new gain-of-structure epitopes generated by non-covalent forces underlying specific aggregate morphologies, such as Abeta oligomers in Alzheimer's disease (15).

Loss of structure can occur when a structured domain of a protein "loosens" revealing a linear sequence that can be recognized by an antibody generated against the free peptide sequence. We have developed rational methods for predicting disease-specific epitopes caused by loss of structure, for which the outputs are sequences of linear peptides that go from structured to unstructured in the misfolding protein, against which antibodies can be generated and screened against the target peptide in the context of the protein misfolded in disease. For example, we identified misfolding-specific epitopes in SOD1 by both hypothesis generation and computational approaches, to block the toxicity and prion-like propagation of SOD1 misfolding in amyotrophic lateral sclerosis (16,17).

Theoretical risk of iatrogenic transmission

There is no doubt that true infectious prions can be detected in the blood and are competent to transmit disease (18); indeed, for variant CJD there have been five well-documented transmissions of disease through blood or blood products (19). However, the presence of protein misfolding seeds in non-prion propagated protein misfolding neurodegenerative diseases has been less clear. Only one journal report of blood Abeta oligomers in Alzheimer's disease has been published (20).

A few scientific reports (including scientific presentations at meetings and patent applications) suggest that transmission of Alzheimer's disease pathology can occur through bloodborne transmission in experimental animals. Alpha synuclein oligomers have been detected in the plasma of patients with Parkinson's disease, but are also observed in similar levels in normal controls, suggesting that the detected analyte is not pathogenic (21). No reports exist of SOD1 or TDP43 oligomers in the peripheral blood or serum, perhaps due to inadequate sensitivity and/or the transmission of bloodborne seeding activity through alternate pathways, such as exosomes (17). Interestingly, a single report shows that individuals injected with cadaveric growth hormone have a higher incidence of amyotrophic lateral sclerosis, but not Alzheimer's disease or Parkinson's disease (22). Thus, although a theoretical risk may pertain to iatrogenic transmission of neurodegenerative diseases, little experimental and epidemiological work supports this.

Conclusions

A new era in diagnosis and treatment of propagated protein misfolding diseases is upon us, offering many opportunities to treat neurodegenerative diseases that were previously untreatable or poorly treatable. Specific immunotherapies to block propagation and toxicity of misfolded proteins hold potential for treatment. The spectre of iatrogenic transmission of amyotrophic lateral sclerosis, Alzheimer's disease or Parkinson's disease and other neurodegenerative syndromes is a theoretical risk, but there is little to no support for seeding species of non-prion propagated protein misfolding diseases being detectable in peripheral blood, or experimental paradigms demonstrating this unsettling prospect at the present time.

Conflict of interest

Dr. Neil Cashman is the Canada Research Chair in Neurodegeneration and Protein Misfolding Diseases and is the Founder and Chief Scientific Officer of ProMIS Neurosciences Inc.

References

(1) Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science. 1982;216(4542):136–44.

(2) Moreno-Gonzalez I, Soto C. Misfolded protein aggregates: Mechanisms, structures and potential for disease transmission. Semin Cell Dev Biol. 2011 Jul;22(5):482–7.

(3) Kane MD, et al. Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. J Neurosci. 2000;20(10):3606–11.

(4) Luk KC, et al. Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. J Exp Med. 2012;209(5):975–86.

(5) Watts JC, Giles K, Oehler A, et al. Transmission of multiple system atrophy prions to transgenic mice. Proc Natl Acad Sci USA. 2013;110(48):19555–60.

(6) Grad LI, et al. From molecule to molecule and cell to cell: Prion-like mechanisms in amyotrophic lateral sclerosis. Neurobiol Dis. 2015 May;77:257–65.

(7) Brettschneider J, Arai K, Del Tredici K, et al. TDP-43 pathology and neuronal loss in amyotrophic lateral sclerosis spinal cord. Acta Neuropathol. 2014;128(3):423–37.

(8) Frost B, Diamond MI. Prion-like mechanisms in neurodegenerative diseases. Nat Rev Neurosci. 2010;11(3):155–9.

(9) Watts JC, Condello C, Stohr J, et al. Serial propagation of distinct strains of Abeta prions from Alzheimer's disease patients. Proc Natl Acad Sci USA. 2014;111(28):10323–8.

(10) Stohr J, Condello C, Watts JC, et al. Distinct synthetic Abeta prion strains producing different amyloid deposits in bigenic mice. Proc Natl Acad Sci USA. 2014;111(28):10329–34.

(11) Guo JL, Covell DJ, Daniels JP, et al. Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. Cell. 2013;154(1):103–17.

(12) Ayers JI, Fromholt S, Koch M, et al. Experimental transmissibility of mutant SOD1 motor neuron disease. Acta Neuropathol. 2014;128(6):791–803.

(13) Sanders DW, Kaufman SK, DeVos SL, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron. 2014;82(6):1271–88.

(14) Guest WC, Silverman JM, Pokrishevsky E, et al. Generalization of the prion hypothesis to other neurodegenerative diseases: An imperfect fit. J Toxicol Environ Health A. 2011;74(22–24):1433–59.

(15) Silverman J, Gibbs E, Rezaei H, et al. An immunological epitope specific for toxic oligomeric Abeta in Alzheimer's disease. Alzheimer's & Dementia: The Journal of the Alzheimer's Association. 2012;8(4):P625.

(16) Grad LI, Yerbury JJ, Turner BJ, et al. Intercellular propagated misfolding of wild-type Cu/Zn superoxide dismutase occurs via exosome-dependent and -independent mechanisms. Proc Natl Acad Sci USA. 2014;111(9):3620–5.

(17) Grad LI, Guest WC, Yanai A, et al. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. Proc Natl Acad Sci USA. 2011;108(39):16398–403.

(18) Wood H. Prion disease: New techniques developed for prion detection in blood and cerebrospinal fluid. Nat Rev Neurol. 2011;7(4):183.

(19) Lefrere JJ, Hewitt P. From mad cows to sensible blood transfusion: The risk of prion transmission by labile blood components in the United Kingdom and in France. Transfusion. 2009;49(4):797–812.

(20) Zhang J, Peng M, Jia J. Plasma amyloid-beta oligomers and soluble tumor necrosis factor receptors as potential biomarkers of AD. Curr Alzheimer Res. 2014 May;11(4):325–31.

(21) Park MJ, Cheon SM, Bae HR, et al. Elevated levels of alpha-synuclein oligomer in the cerebrospinal fluid of drug-naive patients with Parkinson's disease. J Clin Neurol. 2011 Dec;7(4):215–22.

(22) Irwin DJ, et al. Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone. JAMA Neurol. 2013 Apr;70(4):462–8.

ID News: Protein misfolding

Nakamura T, Prikhodko OA, Pirie E, Nagar S, Akhtar MW, Oh CK, et al. **Aberrant protein S-nitrosylation contributes to the pathophysiology of neurodegenerative diseases**. Neurobiol Dis. 2015 Mar 18. pii: S0969-9961(15)00089-3. doi: 10.1016/j.nbd.2015.03.017. [Epub ahead of print] *(Summary)*
http://www.ncbi.nlm.nih.gov/pubmed/25796565

Nitric oxide (NO) is a gasotransmitter that impacts fundamental aspects of neuronal function... During normal brain function, protein S-nitrosylation serves as an important cellular mechanism that modulates a diverse array of physiological processes, including transcriptional activity, synaptic plasticity, and neuronal survival. In contrast, emerging evidence suggests that aging and disease-linked environmental risk factors exacerbate nitrosative stress via excessive production of NO. Consequently, aberrant S-nitrosylation occurs and represents a common pathological feature that contributes to the onset and progression of multiple neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's diseases. In the current review, we highlight recent key findings on aberrant protein S-nitrosylation showing that this reaction triggers protein misfolding, mitochondrial dysfunction, transcriptional dysregulation, synaptic damage, and neuronal injury... We speculate that intervention to prevent these aberrant S-nitrosylation events may produce novel therapeutic agents to combat neurodegenerative diseases.

Goñi F, Mathiason CK, Yim L, Wong K, Hayes-Klug J, Nalls A, et al. **Mucosal immunization with an attenuated Salmonella vaccine partially protects white-tailed deer from chronic wasting disease**. Vaccine. 2015 Jan 29;33(5):726-33. doi: 10.1016/j.vaccine.2014.11.035. *(Summary)*
http://www.ncbi.nlm.nih.gov/pubmed/25539804

Prion disease is a unique category of illness, affecting both animals and humans, in which the underlying pathogenesis is related to a conformational change of a normal, self-protein called PrP(C) (C for cellular) to a pathological and infectious conformer known as PrP(Sc) (Sc for scrapie). Bovine spongiform encephalopathy (BSE), a prion disease believed to have arisen from feeding cattle with prion contaminated meat and bone meal products, crossed the species barrier to infect humans. Chronic wasting disease (CWD) infects large numbers of deer and elk, with the potential to infect humans. Currently no prionosis has an effective treatment. Previously, we have demonstrated we could prevent transmission of prions in a proportion of susceptible mice with a mucosal vaccine. In the current study, white-tailed deer were orally inoculated with attenuated Salmonella expressing PrP, while control deer were orally inoculated with vehicle attenuated Salmonella. Once a mucosal response was established, the vaccinated animals were boosted orally and locally by application of polymerized recombinant PrP onto the tonsils and rectal mucosa. The vaccinated and control animals were then challenged orally with CWD-infected brain homogenate. Three years post CWD oral challenge all control deer developed clinical CWD (median survival 602 days), while among the vaccinated there was a significant prolongation of the incubation period (median survival 909 days; p=0.012 by Weibull regression analysis) and one deer has remained CWD free both clinically and by RAMALT and tonsil biopsies. This negative vaccinate has the highest titers of IgA in saliva and systemic IgG against PrP. Western blots showed that immunoglobulins from this vaccinate react to PrP(CWD). We document the first partially successful vaccination for a prion disease in a species naturally at risk.